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# THE REGENERATION OF THE BLOOD

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# THE REGENERATION OF THE BLOOD.

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PLATES XXXI, XXXII, AND XXXIII.

THE experiments and observations to be described were made with the twofold object of determining the course of the regeneration of the blood in animals which had been subjected to a loss of a part of the blood and of testing the effect upon blood regeneration of the transfusion of an artificial serum.

The classical observations and experiments of Lyon (1) * and Otto (2) have been the foundation of a large part of the subsequent work in this field. The more exact methods of the present decade open up new problems in the old field and extend its boundaries on all sides. In 1891 Reinert (3) investigated the question of the relation of number of red blood-corpuscles to the amount of hæmoglobin. Last year Koeppe (4) made extended and most careful experiments and observations upon rabbits, from which a part of the blood had been withdrawn. Our experiments differ from Koeppe's in that the withdrawal of blood was followed by an intravenous injection of an artificial serum.

## A. METHODS.

The observations were made upon healthy young dogs of medium size. The experiments upon each animal were introduced with a series of observations upon the normal blood. These were made

* The numbers in parentheses refer to literature at the end of article.

with the hæmatocrit or centrifuge, the hæmometer, the corpuscle-counter, the micrometer, and with the Ehrlich method of staining.

For these observations, the blood was drawn uniformly from the under surface of the ear, which had been prepared by a thorough cleansing with soap and water, with water, and with absolute alcohol. The blood was obtained by making with a sharp, clean lancet a wound large enough to produce in a few seconds a large, free drop of blood.

### 1. THE METHODS OF OBSERVATION.

*a. The Hæmatocrit.*—1. One end of the capillary tube is placed in the fresh, free drop of blood. Sometimes capillary attraction will fill the tube quickly enough, but usually slight suction must be made with the mouth.

2. The tube is adjusted, the instrument set in motion, and maintained at a steady rate of speed for two minutes, at the end of which time the corpuscles are separated from the plasma and the reading is easily made, as the line between the corpuscles and plasma is distinct.

Hedin (5) suggests that the centrifugation be kept up for at least five minutes; but Hedin's hæmatocrit made only six thousand revolutions per minute, and he always diluted the blood with about equal proportions of an approximately isotonic saline solution. Cowe (7) also dilutes the blood. We do not dilute the blood. Our instrument makes ten thousand revolutions per minute. Repeated trial shows that this rate of rotation will so completely separate the morphotic elements from the plasma that increase of the time to two and a half or three minutes makes no appreciable difference in the reading. The proportion of white corpuscles in normal blood is too small to be even approximately estimated by this method.

*Precautions.*—A free drop of blood must be secured. If the parts are squeezed to get the blood, the proportion of plasma will be increased. Schwyzer (6) also calls attention to this precaution. Not more than thirty seconds should elapse from the time the blood is drawn until it is in the hæmatocrit, for the separation must be prac-

tically complete before coagulation sets in or the observation must be repeated. It is scarcely necessary to say that the capillary tube must be perfectly clean.

*b. The Hæmometer (Fleischl's).*—The purpose of this instrument is to estimate the amount of the hæmoglobin, while the hæmatocrit determines the proportion of red corpuscles to plasma.

The capillary tube which accompanies each instrument is readily filled by capillary attraction on holding one end of the tube to the free drop of blood. The blood is washed with distilled water out of the just filled tube into one chamber of the receptacle, which is then filled level full. The second chamber is also filled with distilled water. The reading is made in a darkened place with the help of artificial light.

*Precautions.*—As with the hæmatocrit, so with the hæmometer, the drop of blood must be free, and not more than thirty seconds should elapse from the time the blood is drawn till it is in the receptacle. The blood should be thoroughly mixed with water. The presence of ever so small a clot necessitates the repetition of the observation. In filling the capillary tube the greatest care should be taken to avoid getting blood upon the outside of the tube and to insure the exact filling of the tube, for a very slight error here would make a difference of ten per cent in the readings. The chambers of the receptacle should not be filled full enough to permit the fluid to mix above the partition.

The most enthusiastic admirer of the hæmometer would not contend that the results obtained with it are more than approximate; they may be correct within about five per cent if the observations were made with all of the enumerated precautions and the readings made with the greatest care. Experimentation has shown that several observers may differ as widely as fifteen per cent on the same reading; furthermore, that the same observer may vary more than ten per cent in repeating a reading. Two simultaneous observations, made upon the same animal, with two Fleischl's hæmometers placed side by side and read by the same individual, may differ as much as eight per cent. Busch and Kerr (8) ascribe to Fleischl's hæmometer

an error range of ten per cent, and to Gower's a still greater inaccuracy.

*c. The Corpuscle Counter* (*Zeiss's*).—The blood was mixed with an artificial serum made as follows:

| | |
|---|---|
| Gum-arabic solution (sp. gr., 1020)........ | 20 c. c. |
| Sodium-chloride solution (sp. gr., 1020).... | 30 " |
| Sodium-sulphate solution (sp. gr., 1020).... | 30 " |

Mix and filter.

Winternitz (9) advises the dilution of the blood with potassium bichromate (2.5 per cent) for counting the red blood-corpuscles, and with acetic acid (0.1 per cent) for counting the white blood-corpuscles. The above formula advised by Landois answers our purpose well.

*Precautions.*—The dilution of the blood in the " mixer " must be made with the greatest precision, because the microscopical readings are to be multiplied by 400,000. Any error made in the dilution will be multiplied by that factor. The blood must be completely removed from the outside of the end of the mixing tube before dipping it into the artificial serum, otherwise a part of this blood will be drawn into the tube and thus vitiate the results.

When shaking the bulb to effect the complete mixture of the blood with the diluting fluid the rubber tube, which is connected with the upper end of the " mixer," should be drawn tightly across the end of the glass tube to prevent a part of the liquid being thrown out by the centrifugal force. If the mixing tube becomes clogged with a clot, a strong alkali, such as caustic potash (ten to twenty per cent), must be used to dissolve it. Before the tube can be used again it must be freed from every vestige of the alkali, otherwise some of the red blood-corpuscles will be dissolved and disappear.

In cleaning the " mixer " *draw distilled water through it;* if the water be drawn into the bulb, and then forced out by blowing into the rubber tube, saliva may pass into the mixer. In the same way and for the same reasons draw absolute alcohol through the bulb after the latter has been rinsed with distilled water. Dry the tube by *drawing* air through it.

*d. The Micrometer.*—The micrometer is used to determine the diameter of the corpuscles. Many attempt to measure corpuscles which are suspended in a diluted plasma, but Hamburger (10) says that "into whatever solution one may bring the red corpuscles—isotonic, hyperisotonic, or hypisotonic salt or sugar solutions, or diluted serum—the corpuscles always lose the biconcave shape and suffer thereby a lessening of their diameter. If returned to normal plasma, they regain the normal form." After repeated attempts to so adjust our diluting fluid as to lead to no change in the shape of the disks, we adopted the following method, which is very practical, especially for the determination of relative values where absolute values are of secondary importance: Spread upon a cover-glass the thinnest possible film of blood. Exposure to the air will dry it in a few moments. Lay the cover, blood-side down, upon a slide; place under the microscope, and measure corpuscles taken at random from the field, avoiding, however, those which may have become accidentally distorted in the process given above. The average of not fewer than fifty corpuscles should be taken.

The accuracy of the results depends upon the accuracy of the determination of the value of the divisions in the micrometer and upon the accuracy of the measurement of individual corpuscles. Besides the errors of observation incident to the difficulty of getting an absolutely sharp focus of both the corpuscles and the micrometer, and of bringing the micrometer to a point absolutely tangent to the corpuscle—besides these necessary sources of error in observation, there is always a distinct personal error.

*e. Ehrlich's Method of staining Blood, as given by Rieder* (11), *of Munich.*—(1) Place a very small drop of blood upon a perfectly clean cover-glass. Place upon this another cover-glass; press the two together until the blood is in a very thin film; then quickly draw the glasses apart, and expose to the air for a minute or two.

(2) Place the specimens in a drying-oven, and gradually raise the temperature to between 110° C. and 120° C., which temperature is maintained for two hours.

(3) Make an eosin stain according to the following formula:

| | |
|---|---|
| Pure glycerin........................ | 95 c. c. |
| Carbolic acid......................... | 5 " |

Saturate this mixture—or rather 10 cubic centimetres of it—with eosin by stirring the eosin in with a glass rod. To insure saturation there should be an excess of undissolved eosin in the bottom of the receptacle.

(4) Place one or two drops of this saturated eosin-carbol-glycerin upon a slide; invert one of the cover-glass specimens upon the eosin, and leave at room temperature for at least two hours.

(5) Rinse with distilled water.

(6) Treat one minute with hæmatoxylin (Boehmer's or Delafield's).

(7) Rinse again with distilled water, and dry in the air.

(8) Mount in balsam.

*Precautions.*—The cover-glasses used must be absolutely clean. The blood must be spread in a very thin film upon the cover-glass. The staining with hæmatoxylin must be carefully timed.

*General Precaution.*—In all of these different methods of examining the blood three things must be observed: 1. Absolute cleanliness. 2. Exactness in all readings and determinations. 3. Rapidity of work to avoid too early clotting of blood.

## 2. THE WITHDRAWAL OF BLOOD.

*a. Preparation.*—The dog was weighed, fixed to the operating board, and anæsthetized.

The femoral region was chosen as the point for operation, and was prepared according to the rules of aseptic surgery.

*b. Operation.*—An incision is made through the skin over the femoral vessels, whose superficial location facilitates their use for this operation. The artery and vein are dissected to the extent of an inch and a half. A glass cannula, which is provided with a few inches of rubber tubing, is inserted into the artery with the opening toward the heart. The blood flows in jets into a weighed receptacle. A similar cannula, provided with a rubber tube, is inserted into the vein.

This cannula is connected by the rubber tube to a 100-cubic-centimetre burette.

*c. Transfusion.*—Following the American Text-book of Surgery, the word "transfusion" is here used to include the injection into the circulatory system of artificial serum or of normal saline solution. In these experiments we used an artificial serum made after the following formula:

| | |
|---|---|
| Cane sugar........................ | 35 grammes. |
| Common salt...................... | 6 " |
| Sodium hydrate.................... | 0.05 gramme. |

Distilled water, a quantity sufficient to make 1,000 grammes.

The fluid was sterilized by maintaining it at a boiling temperature for three daily periods of one hour each.

The sterilized serum at a temperature of 40° C. was poured into the burette and allowed to flow slowly into the vein under the influence of the hydrostatic pressure. It is needless to say that the necessary precautions were taken to prevent the introduction of air bubbles into the vein. The blood was withdrawn and the fluid injected at the same time. At least thirty minutes should be consumed in the withdrawal of the blood and the transfusion of the fluid, otherwise there will be too rapid a change in the blood pressure, or possibly rather in the quality of the blood, inducing serious symptoms. The amount of blood taken from the dogs was equal to five per cent of the body weight, and the amount of artificial serum injected was equal to six per cent of the body weight, or twenty per cent more than the amount of blood withdrawn.*

*d. After-treatment.*—The wound was closed and aseptically dressed. The dog was kept in a warm room and fed on nourishing broths for three days, after which time he was treated as were the normal dogs.

All of the dogs made a good recovery except one, which had a secondary hæmorrhage on the eighth day, due to his repeated tearing

*In the case of Dog D the amount of blood withdrawn equalled four per cent of the body weight and no transfusion followed.

off of the dressings. In a subsequent operation the vessels of the neck, rather than those of the thigh, were chosen for the operation, with the result that the dressings, being inaccessible to the dog's teeth, were left undisturbed.

## B. Results.

### 1. The tabulated results.

The observations made are given in the following tables.

Table I. Dog A.

| 1 Date. | 2 Hæmatocrit. | 3 Counter. (No. in millions.) | 4 Hæmometer. | 5 Micrometer. | Remarks. |
|---|---|---|---|---|---|
| Jan. 13th... | 50 | 5·880 | 85 | 5·0$\mu$ | |
| " 15th... | 50 | 5·568 | 85 | 5·0$\mu$ | |
| " 17th... | 46·6 | 5·304 | 85 | 5·8 | The dog had drunk freely of water about 2 hrs. before observation. |
| **Average....** | **48·9** | **5·586** | **85** | **5·3$\mu$** | |
| Jan. 18th... | 21·6 | 2·490 | 35 | 5·65 | Operation. |
| " 20th... | 22·5 | 2·960 | 40 | 5·45 | Neutrophiles increased. |
| " 22d.... | 23·6 | 1·188 | 45 | 5·45 | " " |
| " 24th... | 26·3 | 1·308 | 47 | 5·2 | |
| **Average....** | **23·5** | **2·238** | **41·7** | **5·4+** | |
| Jan. 27th... | 20 | ..... | 35 | 6·0 | Secondary hæmorrhage. |
| " 29th... | 22·5 | ..... | 40 | 6·0 | Corpuscles very variable in size. |
| " 31st... | 24·2 | ..... | 40 | 6·0 | " " " " |
| **Average....** | **22·5** | **[2·142]** | **38·3** | **6·0** | |
| Feb. 3d..... | 25 | 2·010 | 40 | 6·2 | |
| " 5th.... | 30 | 1·800 | 45 | 6·2 | |
| " 7th.... | 29 | 2·232 | 45 | 6·5 | |
| **Average....** | **28** | **2·014** | **43·3** | **6·3** | |
| Feb. 10th... | 30 | 2·180 | 47 | 6·6 | |
| " 12th... | 33 | 2·228 | 55 | 5·9 | Corpuscles irregular in shape. |
| " 14th... | 35 | 2·160 | 55 | 6·5 | |
| **Average....** | **32·6** | **2·182** | **52·3** | **6·3** | |
| Feb. 17th... | 33 | 3·684 | .... | .... | |
| " 19th... | 34 | 3·440 | 60 | 6·7 | |
| " 21st... | 33 | 4·580 | 60 | 5·7 | |
| **Average....** | **33·3** | **3·901** | **60** | **6·2** | |
| Feb. 24th... | 35 | 3·480 | 75 | 6·2 | |
| " 26th... | 43 | 5·304 | 75 | 6·3 | |
| " 28th... | 45 | 3·216 | 75 | .... | |
| **Average....** | **41** | **3·996** | **75** | **6·25** | Neutrophiles less numerous. |
| March 2d... | 44 | 5·000 | 100 | .... | |
| " 4th .. | 42 | 6·000 | 85 | .... | |
| " 6th .. | 49 | 6·160 | 85 | 6·6 | Corpuscles regular. |
| **Average....** | 45 | **5·720** | **90** | **6·6** | |

Note from Column 1 of Table I that the observations were made upon alternate days, except Sunday, and that the averages usually

represent periods of one week. The date of the operation is recorded in Column 6 under "Remarks." On the day of the operation the observations were made immediately after the withdrawal of blood.

At first thought the observations would seem to be too far apart to show certain important changes which occur the first day after hæmorrhage. Both Otto (2) and Koeppe (4) have recorded and discussed these changes. Their experiments did not involve the intravenous injection of a diluting fluid, and therefore twenty-four to forty-eight hours would be required to so dilute the blood with tissue plasma as to reduce the hæmoglobin and number of red blood-corpuscles to a minimum; in our experiments the injection of an artificial serum reduced the hæmoglobin-content and the number and volume of red blood-corpuscles at once to the minimum, so that the observations taken immediately after the operation always represent the minimum. From this point on the change is one of gradual improvement.

The hæmatocrit observations are recorded in percentages. From this column it will be seen that the average proportion of corpuscles in the normal blood of Dog A is 48.9 per cent, and that immediately after the operation the proportion was reduced to 21.6 per cent. From this point it gradually rises for eight days, when the secondary hæmorrhage brings it back to twenty per cent. From this time the gain, as indicated in the average, is continuous and fairly uniform.

The results obtained with the corpuscle-counter are recorded in Column 3. The observations are recorded in millions. Note an approximate parallelism between the results of the counter and those of the hæmatocrit. That the parallelism is only approximate may be accounted for as follows: The total volume of the corpuscles as observed with the hæmatocrit depends not only upon the number of corpuscles, but also upon the size of the individual corpuscles.

The hiatus in this series of observations (January 27th to 31st) was due to the accidental contamination of the mixing tube and the fluid, used in diluting the blood in the mixer, with the caustic potash, which had been used to clean the mixer. The average here given was interpolated.

The hæmometer readings, which appear in Column 4, are given

on an arbitrary basis of 100, assumed by Fleischl, and approximately representing normal blood. It will be noticed that the results obtained with the hæmometer are closely parallel to those obtained with hæmatocrit and counter. The diameter of the corpuscles is recorded in Column 5 in micromillimetres.

TABLE II. DOG B.

| DATE. | 2 Hæmato-crit. | 3 Counter. (No. in millions.) | 4 Hæmom-eter. | 5 Microm eter. | 6 Remarks. |
|---|---|---|---|---|---|
| Jan. 20th... | 54·8 | 6·280 | 85 | 5·1μ | |
| " 22d.... | 51·4 | 6·880 | 70 | 5·25 | |
| " 24th... | 53·3 | 6·436 | 95 | 5·25 | |
| **Average....** | **53·1** | **6·532** | **83·3** | **5·17** | |
| Jan. 25th... | 33·3 | .... | 55 | 5·1 | Operation for withdrawal of blood. |
| " 27th... | 29·7 | 2·796 | 45 | 5·25 | |
| " 29th... | 29·7 | 2·996 | 50 | 5·8 | |
| **Average....** | **30·9** | **2·896** | **50** | **5·5** | |
| Jan. 31st... | 34·5 | 3·480 | 53 | 6·0 | |
| Feb. 3d..... | 23·0 | 1·760 | 40 | 6·5 | |
| " 5th.... | 27 | 2·480 | 45 | 8·0 | Neutrophiles numerous. |
| **Average....** | **28·2** | **2·573** | **49·3** | **6·8** | Corpuscles irregular in shape. |
| Feb. 7th... | 31 | 2·144 | 45 | 6·4 | |
| " 10th... | 33 | 3·560 | 50 | 6·2 | |
| " 12th... | .... | 4·160 | 60 | 6·5 | |
| **Average....** | **32** | **3·288** | **52** | **6·33** | |
| Feb. 14th... | 38 | 3·030 | 60 | 6·5 | |
| " 17th... | 38 | 2·552 | 65 | .... | Neutrophiles. |
| " 19th... | 33·3 | 3·408 | 65 | 5·9 | |
| **Average....** | **36·3** | **2·993** | **63·3** | **6·2** | |
| Feb. 21st... | 36 | 4·688 | 65 | 6·1 | |
| " 24th... | 40 | 7·080 | 90 | 4·6 | |
| " 26th... | 45 | 6·380 | 80 | 6·5 | |
| **Average....** | **40·3** | **6·049** | **78** | **5·7** | |
| Feb. 28th... | 43 | 5·496 | 100 | .... | |
| March 1st... | 50 | 7·880 | 100 | .... | |
| " 4th.. | 49 | 5·000 | 85 | .... | |
| **Average....** | **47·3** | **6·125** | **95** | ... | |
| March 6th... | 54 | 5·288 | 90 | 6·4 | Neutrophiles disappear. |
| " 9th... | 52 | 6·880 | 105 | 6·5 | |
| " 11th.. | 53 | 8·000 | 110 | 6·5 | |
| **Average....** | **53** | **6·722** | **100+** | **6·5** | |

TABLE III. DOG C.

| DATE. | 2 Hæmato-crit. | 3 Counter. (No. in millions.) | 4 Hæmom-eter. | 5 Microm-eter. | 6 Remarks. |
|---|---|---|---|---|---|
| April 8th.. | 35 | 5·560 | 70 | 6·7μ | |
| " 10th.. | 35·6 | 5·290 | 70 | 5·7 | |
| " 13th.. | 37·1 | 5·176 | 60 | 5·85 | |
| **Average....** | **35·9** | **5·342** | **66·6** | **6·1** | |

TABLE III. DOG C—(*Continued*).

| 1 DATE. | 2 Hæmatocrit. | 3 Counter. (No. in millions.) | 4 Hæmometer. | 5 Micrometer. | 6 Remarks. |
|---|---|---|---|---|---|
| April 14th.. | 43·6 | 5·000 | 75 | 6·0 | |
| " 16th.. | 40·7 | 5·944 | 70 | 6·5 | |
| " 18th.. | 37·0 | 5·120 | 85 | 5·45 | |
| **Average....** | **40·4** | **5·336** | **76·6** | **6·0** | |
| April 21st.. | 40 | 5·211 | 75 | 6·0 | |
| " 23d... | 43·3 | 5·362 | 80 | 6·0 | |
| " 25th.. | 41·9 | 6·420 | 80 | 6·0 | |
| " 28th.. | 41 | 4·748 | 75 | 6·0 | |
| **Average....** | **41** | **5·435** | **77·5** | **6·0** | |
| May 1st.... | 27·7 | 3·620 | 50 | 5·8 | Withdrawal of blood. |
| " 4th.... | 24 | 4·000 | 50 | 6·0 | |
| " 6th.... | 22·5 | 2·652 | 50 | 6·2 | |
| **Average....** | **24·7** | **3·424** | **50** | **6·0** | |
| May 8th... | 26·0 | 3·216 | 52 | 6·9 | |
| " 10th... | 28·2 | 3·748 | .. | 6·5 | |
| " 12th... | 30·3 | 4·280 | 60 | 6·5 | |
| **Average....** | **26·2** | **3·718** | **56** | **6·6** | |
| May 15th... | 32·5 | 4·990 | 60 | 6·4 | |
| " 19th... | 34·0 | 4·296 | 60 | 6·0 | |
| " 21st... | 40·9 | 5·008 | 75 | 6·6 | |
| **Average....** | **35·8** | **4·764** | **65** | **6·3** | |

TABLE IV. DOG D.

| 1 DATE. | 2 Hæmatocrit. | 3 Counter. (No. in millions.) | 4 Hæmometer. | 5 Micrometer. | 6 Remarks. |
|---|---|---|---|---|---|
| May 4th.... | 46 | 7·000 | 80 | 6·4 | |
| " 6th.... | 40 | 5·000 | 80 | 6·0 | |
| " 8th.... | 40·6 | 5·248 | 80 | 5·7 | |
| **Normal.....** | **42·2** | **5·749** | **80** | **6·0** | |
| May 10th... | .... | ..... | .. | ... | Withdrawal of 40 per cent of total volume of blood not followed by transfusion. |
| " 10th... | 32·3 | 5·120 | 70 | 6·0 | |
| " 12th... | 31·0 | 3·964 | 50 | 6·0 | |
| " 14th... | 28·0 | 4·264 | 55 | 6·3 | |
| " 19th... | 30·6 | 4·624 | 70 | 6·5 | |

Tables II and III are similar to Table I, and need no separate explanation. Table IV gives the results of observations on Dog D. This experiment differs from the preceding ones in that the withdrawal of blood was not followed by the transfusion of artificial serum. The record shows that the decrease in the readings after the operation was not so sudden or so great as was the case in the other dogs; further, that the readings continued to decrease for several days, reaching a minimum on the third or fourth day after the opera-

tion, while all of the other dogs reached their minimum immediately after the withdrawal of the blood. This accounts for the apparent retardation of the regeneration of the blood in the case of Dog D.

Dog D was in good condition, and seemed to be perfectly normal during the preliminary observations (May 4th to 10th). His recovery from the loss of blood was not so rapid as was that of the other dogs. The reason for this is apparent; the volume of the blood had been decreased by about one half, and had not been replenished by the transfusion of artificial serum. Four days after the operation the dog showed all the symptoms which follow a severe hæmorrhage, while the other dogs were on the second day quite free from those symptoms.

A comparison of the number of corpuscles in Dog D immediately after the withdrawal of blood with that observed in the other dogs at the same period shows that, while there was a marked falling off in all of the others, in Dog D there was only a slight decrease. A moment's reflection makes it evident that the dilution of the blood with the transfusion fluid will markedly reduce the number of red blood-corpuscles per unit volume. Now, there being, in the case of Dog D, no transfusion of an artificial serum, the blood would be diluted only by the lymph plasma taken up from the tissues. The dilution would naturally be small in amount, as attested by the slight difference, before and after the operation, not only in the number of corpuscles, but also in the volume and in the amount of hæmoglobin.

The fact that our Dog D did not reach a minimum until the fourth day instead of the first or second, as was the case with the rabbits on which Otto and Koeppe experimented, may probably be accounted for by the severer loss which Dog D suffered. Béchamp (12) says that dogs will stand a loss of blood equal to three to four per cent of the body weight; Huenerfauth (13) gives three and a half to four and a half per cent. The limit undoubtedly varies with different individuals.

The symptoms shown by Dog D during the four days subsequent to the operation made it evident that four per cent was practically the limit for that dog. That the fall to minimum should in

this case continue for four days, while in the case where blood equal to only 2.2 per cent of the body weight is withdrawn—see Limbeck's (14) experiment—the minimum should be not much less than the normal and be reached during the first day, is a result to be expected.

In order to get a general view of the results from the three similar experiments on Dogs A, B, and C, the averages from Tables I, II, and III are combined in Table V in such a manner as to make the observations taken immediately after the operation fall in the same column headed "Operation." The observations on Dog C were taken for three weeks before the operation, while on Dogs A and B they were taken for only one week before the operation. This necessitates the interpolation of the averages as given in Columns 4 and 5.

Table V. Combining the Averages of Dogs A, B, C.

| No. | Instruments. | Dog. | Normal I. | Normal II. | Normal III. | Operation 1. | Regeneration of the blood. 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I. | Hæmatocrit. | A. | .... | .... | 48·8 | 23·5 | 22·5 | 28·4 | 32·6 | 33·3 | 41 | 45 | ... |
| | | B. | .... | .... | 53·1 | 30·9 | 28·2 | 32·0 | 36·3 | 40·3 | 47 | 56 | ... |
| | | C. | 35·1 | 40·4 | 41·0 | 24·7 | 26·2 | 35·8 | ... | ... | ... | ... | ... |
| | | **Av.** | **[37·1]** | **[46·9]** | **47·6** | **26·3** | **25·6** | **32·1** | **34·4** | **36·8** | **44** | **50** | ... |
| II. | Counter. | A. | .... | .... | 5·58 | 2·24 | [2·14] | 2·01 | 2·18 | 3·90 | 3·99 | 5·72 | 7·19 |
| | | B. | .... | .... | 6·53 | 2·90 | 2 57 | 3·28 | 2·99 | 6·04 | 6·12 | 6·72 | ... |
| | | C. | 5·34 | 5·33 | 5·43 | 3·42 | 3·74 | 4·76 | [4·65] | ... | ... | ... | ... |
| | | **Av.** | **[5·71]** | **[5·73]** | **5·81** | **2·85** | **2·82** | **3·35** | **3·27** | **4·97** | **5·01** | **6·22** | **7·19** |
| III. | Hæmometer. | A. | .... | .... | 85 | 41·7 | 38·3 | 43·3 | 52·3 | 60 | 75 | 90 | 95 |
| | | B. | .... | .... | 83·3 | 50·0 | 49·3 | 52·0 | 63·3 | 78 | 95 | 100 | 100 |
| | | C. | 66·6 | 76·6 | 77·5 | 50·0 | 56·0 | 65·0 | ... | ... | ... | ... | ... |
| | | **Av.** | **[77·3]** | **[80·9]** | **81·9** | **47·0** | **47·9** | **53·4** | **57·8** | **69** | **85** | **95** | **97** |
| IV. | Micrometer. | A. | .... | .... | 5·2 | 5·4 | 6·0 | 6·3 | 6·3 | 6·2 | 6·2 | 6·6 | 6·5 |
| | | B. | .... | .... | 5·2 | 5·5 | 6·8 | 6·3 | 6·2 | 5·7 | ... | 6·5 | 6·5 |
| | | C. | 6·1 | 5·9+ | 6 0 | 5·9 | 6·6 | 6·3 | ... | ... | ... | ... | ... |
| | | **Av.** | **[5·53]** | **[54·3]** | **5·45** | **5·6** | **6·5** | **6·3** | **6·25** | **6·0** | **6·2** | **6·55** | **6·5** |

This table shows that the regeneration requires several weeks, and that the process is more rapid in the third and the fourth weeks after the operation than in the first and second weeks.

## 2. Variations of the blood within physiological limits.

By reference to Table III it will be seen that Dog C was under observation for three weeks before the withdrawal of blood, and that

during this time there was quite a variation in the observations made. During the first week there was an increase in the volume and number of the red blood-corpuscles, as shown by the hæmatocrit and counter. The next week the observations vary, but the average for the week is higher than for the previous one. In the third week the observations vary slightly, with an average still higher than that of the second week.

By referring to the weekly averages for the hæmatocrit, counter, and hæmometer, it will be seen that there is a close parallelism in the results of these three methods of observation. But in comparing the daily results they are found to vary widely. It is, of course, possible that this variation may be accounted for on the ground of errors in observation, though the greatest care was taken to reduce such errors to a minimum, as is apparent from the description of methods and precautions. Dogs B, C, and D were taken from the streets, and all seemed to be healthy and in fairly good condition; yet the tables show that they varied greatly as to the quality of the blood. The variation, according to the hæmatocrit, was from thirty-five per cent in Dog C to 54.8 per cent in Dog B. The counter showed 5.56 millions of red blood-corpuscles per cubic millimetre in Dog C and seven millions in Dog D. The hæmometer showed seventy per cent in Dog C and eighty-five per cent in Dog B. Within the limits here indicated the cause and course of the variations are probably physiological. On January 17th, for instance, Dog A drank freely of water. The variations found in the blood on that day would seem to be fully accounted for by the dilution of the plasma, occasioned by the increased imbibition of water. Incident to this dilution there would be relative decrease in the volume of the red blood-corpuscles, relative decrease in the number of corpuscles per unit volume. One would expect also a relative decrease in percentage of hæmoglobin. The latter point was not, however, confirmed. The dilution of the plasma would cause a decrease in its specific gravity, which may account for the increased size of the corpuscles on that day, as shown by the micrometer. But these are only suggestions as to the possible causes.

This subject—*the character, extent, and cause of the variation, within physiological limits, of the quantity and quality of the blood*—is urgently calling for extended investigation. It is the plan of the writers to continue the investigation on the lines marked out in this paper.

The effect of change of altitude has already been investigated by Jaruntowsky and Schroeder (15), Koeppe (16), Siegfried (17), Reinert (18), Grawitz (19), and others; while Czerny (20) and Winternitz (9) have experimented with the changes in the blood induced by change of temperature.

### 3. THE COURSE OF THE REGENERATION OF THE BLOOD.

Regeneration as discussed in the present paper refers especially to the restitution of the whole volume of blood to a quantitatively normal condition. We shall not at present attempt to add to the knowledge of the histogenesis and morphological regeneration of the morphotic elements of the blood—a field already most thoroughly covered by Aquisto (21), Timofejewsky (22), Neumann (23), Vulpian (24), Löwit (25), Engel (26), Zenoni (27), and others.

In order to get a clearer picture of the course of blood regeneration recourse has been had to the graphic method. Attention is invited to Plates XXXI to XXXIII. In these curves the abscissa is divided into six to eleven segments, each representing a period of one week, while the ordinate is divided into ten segments representing the abstract units 1 to 10.

In locating any observation—or rather average—upon these plates the initial digit of the number determines the ordinate of the point, while the time of the observation or average determines the abscissa of the point. Let us take a concrete example: Plate XXXI, Dog A, is derived from the averages in Table I. According to our notation, the first average taken (January 17th) will fall on the first division of the abscissa. The hæmatocrit average—4.89 per cent—will have an ordinate greater than 4 and less than 5—i. e., about 4.9, as nearly as one can locate it on so small a space. In the same way the number of corpuscles per cubic millimetre—5.586 millions—will have an

abscissa of 1 and an ordinate of about 5.6. The averages taken on the 21st of February would all have the abscissa 6, and an ordinate equal respectively to 3.33, 3.9, 6, and 6.2.

Having located the points represented by the averages, one may join these points and thus gain a lucid and correct conception not only of the absolute, but also of the relative changes which the blood underwent. Take, for example, the volume of red blood-corpuscles in Dog A, as represented in the continuous line of Plate XXXI. Note the sudden fall after Period 1, occasioned by the withdrawal of blood. For two periods after the loss of blood there is no essential improvement in the volume of the red blood-corpuscles. With the third period after the withdrawal there begins an increase in the volume of the red blood-corpuscles, which continues, with only minor fluctuations, throughout the remainder of the course of the observations.

Having explained the way in which these curves were traced, and the general interpretation of their rise and fall, we may turn our attention to their special interpretation.

*a. The Relation between Volume of Red Blood-corpuscles and Amount of Hæmoglobin.*—If one compares the hæmatocrit curve—Plate XXXI, Dog A—with the hæmometer curve, one will find that they are practically parallel until Period 5, when they begin to diverge. Inasmuch as this divergence is persistent through four periods and steadily progressive, it requires explanation. The hæmatocrit curve represents *volume* of red blood-corpuscles, while the hæmometer curve represents amount of *hæmoglobin*. The only conclusion that can be drawn is:

*The quantity of hæmoglobin per volume of red blood-corpuscles is not constant.*

*b. The Relation of the Volume of the Red Blood-corpuscles to the Number and Size.*—The volume as recorded by the hæmatocrit varies with the product of two factors: The average volume of the individual corpuscles multiplied by the number of corpuscles per unit volume. This relation may be expressed mathematically by the formula $V \propto v \times n$, in which V equals the total volume. But $v$ can

only be determined through observation of linear dimensions. The diameter only is usually measured.

If all of the dimensions of a red blood disk varied proportionally, then our formula would be $V \propto d^3 \times n$. From this relation we would expect that if the red blood-corpuscles retain their normal volume—i. e., an unvarying diameter, as shown by the micrometer—then the curve representing the number of corpuscles would be practically parallel with the hæmatocrit curve. This is readily shown mathematically by reference to the laws of variables: If $d$ is constant, our formula becomes $V \propto n$, which is equivalent to saying: The curve of Volume is parallel to the curve of Number.

But it is not parallel; it falls below the hæmatocrit curve between the Periods 3 and 5, and after Period 7 rises much above it. This would be mathematically impossible unless there be a variation in the diameter of the corpuscles. And if there is a variation in the diameter of the individual corpuscles, this variation must sustain to the curve of number a reciprocal relation. In other words, if $n$ varies in one direction from V, $d$ must vary in the opposite direction; in the example cited—Plate XXXI, Dog A, Periods 3 to 5—if $n$ becomes relatively less, $d$ should become relatively greater. Now, this phenomenon which the mathematical formula foreshadows is beautifully illustrated and demonstrated in all of the plates. On the other hand, one would expect the micrometer curve to fall after the number curve begins to rise. This may be easily verified in Plate XXXI, Dog B, Plate XXXII, Dog C, and Plate XXXIII, though less easily in Plate XXXI, Dog A, where there is only a slight fall of the micrometer curve in Period 6.

*c. The Relation between the Amount of Hæmoglobin and the Number of Corpuscles.*—That the amount of hæmoglobin does not vary as the volume of the red blood-corpuscles has been demonstrated. A further inspection of the curves shows the hæmometer curves to be practically parallel to their respective number curves. This indicates that *the quantity of hæmoglobin varies as the number of red blood-corpuscles* (Hb $\propto$ $n$), or that each corpuscle has practically the same amount of hæmoglobin, whether it be larger or smaller. One

can easily understand that such would naturally be the case in all variations of size incident to the changes of the specific gravity of the plasma, as was the probable case with Dog A on January 17th.

When, however, the variation is incident to the natural development of a corpuscle, it is more difficult to understand the relations.

Koeppe (4) says that, although it is generally accepted "that in healthy blood the amount of hæmoglobin is, generally speaking, proportional to the number of the red blood-corpuscles," his own observations, as well as those of Reinert (3), would not justify that conclusion. After a careful consideration of the observations of these investigators, the writers feel warranted in reaffirming the tenability of the above very guarded generalization.

Koeppe cites the rapid multiplication of nascent erythrocytes and of microcytes, which increase the number without representing an increase in the Hb. This is unquestionably true, and is demonstrated by the curves on Plate XXXIII, where it will be noted that the number curve is relatively higher during the first three periods after the operation than before or after this time. But this is a special case, and it is not included in the terms of the above formula. Reinert's curves represent single observations and not averages. A large part of the discrepancy between his Hb curves and number curves undoubtedly arises in his failure to neutralize errors of observation by the use of averages.

### 4. THE MICROSCOPICAL OBSERVATIONS.

The well-known observations of Neumann (23), Timofejewsky (22), Zernoni (27), Viola et Jona (28), and others, showing the rapid multiplication of nucleated red blood-corpuscles and of microcytes after hæmorrhage or withdrawal of blood were confirmed in these experiments. There was a moderate increase in eosinophiles, and a very marked increase in neutrophiles during the first week. Besides these confirmatory observations, the experiments afforded a number of suggestive clews, which will be the subject of subsequent experiment.

### 5. On the transfusion of an artificial serum or of a physiological saline solution.

The advantages and disadvantages of this measure as a surgical procedure in cases of severe acute anæmia have been discussed at length by Knoll (29), Ziemssen (31), Feis (32), Ullmann (33), and others. The first classical work in this field was that of Goltz (30), in 1864, who began his experiments upon frogs. He suggested the use of a solution of sodium chloride or some other indifferent solution after severe hæmorrhages, his idea being to avert a profound change in blood pressure. Subsequent experiments show that the change in blood pressure after hæmorrhage is much less marked than had been supposed; it remains true, however, that the regaining of the normal condition and the regeneration of the blood, after a severe hæmorrhage, is much more rapid if there has been a proper intravenous injection. The experiments above described show a very much more rapid recovery of the dogs which were given the benefit of artificial serum transfusion.

## Summary of the Results.

*a.* The blood of apparently normal animals undergoes considerable variations within physiological limits.

*b.* After a loss of blood the regeneration is more rapid if there has been a transfusion of an artificial serum.

*c.* Regeneration after transfusion is less rapid during the first half of the regeneration period than during the second half.

*d.* The regenerative processes once stimulated into activity carry the blood, qualitatively, considerably beyond the established normal. (See Plate XXXIII.) Otto (2) observed a similar phenomenon.

*e.* The quantity of hæmoglobin per volume of red blood-corpuscles is not constant.

*f.* The volume of red blood-corpuscles varies as the product of the average volume of individual corpuscles and the number of corpuscles per unit volume. ($V \propto v \times n$).

*g.* When the number of the corpuscles increases the size decreases, and conversely. ($n \propto \frac{1}{d}$).

*h*. The quantity of hæmoglobin varies in general with the number of red blood-corpuscles per unit volume. (Hb. $\propto n$).

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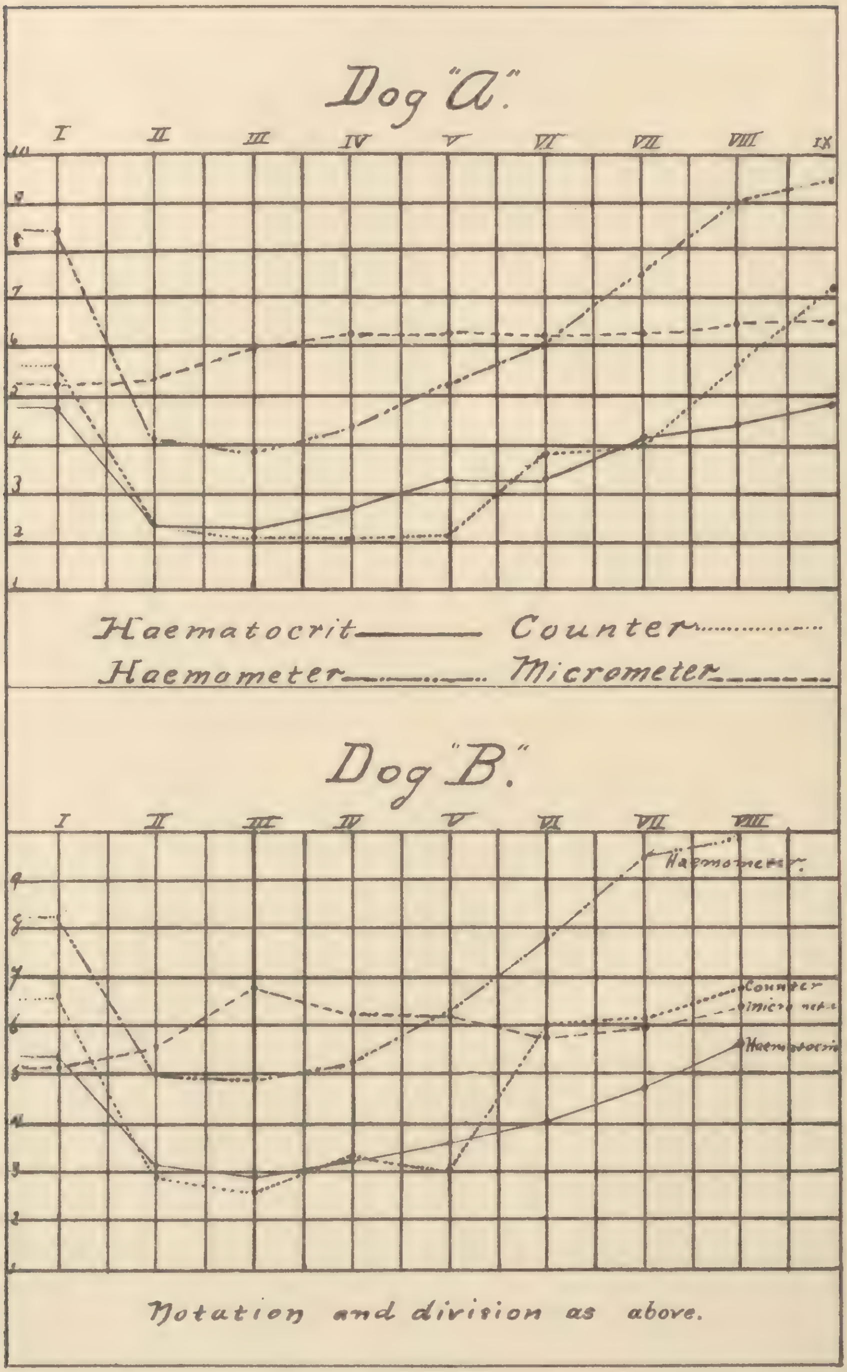
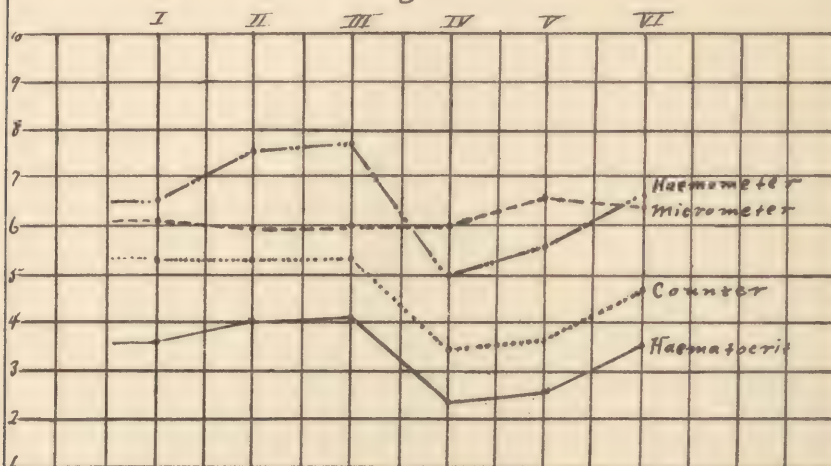
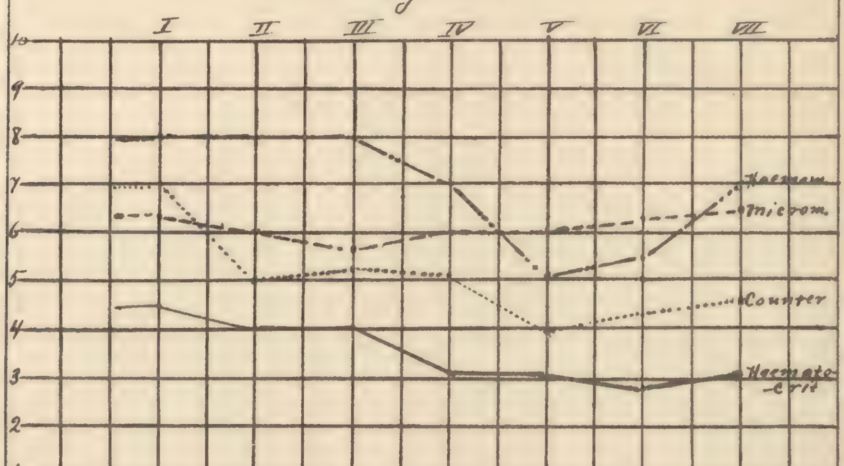

Dog "A."
I
II
III
IV
V
VI
VII
VIII
IX
Haematocrit
Counter
Haemometer
Micrometer
Dog "B."
I
II
III
IV
V
VI
VII
VIII
Haemometer.
Counter
Haematocrit
Notation and division as above.

Dog "C."




Notation as in Plate XXXI.

Dog "D."




Notation as in Plate XXXI.


Composite Curves for Dogs "A," "B" & "C".
Haemometer
Haemotocrit
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
10
9
8
7
6
5
4
3
2
1
Notation as in Plate XXXI.

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## Description of Plates XXXI, XXXII, and XXXIII.

See the text (pp. 15–17).